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Oxidative stress is reduced by the long-term use of vitamin E-coated dialysis filters

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Oxidative stress is reduced by the long-term use of vitamin E-coated dialysis filters.

Background. Oxidative stress during hemodialysis is thought to promote the progression of vascular complications in hemodialysis patients. The protective role of vitamin E as a lipophilic antioxidant against oxidative stress has been widely investigated. Here we investigated the effects of a vitamin E-coated regenerated cellulose hollow fiber dialyzer (CL-EE) on oxidative stress compared with a polysulfone hollow fiber (CL-PS).

Methods. For at least three months before beginning the protocol, 10 nondiabetic (NDM) patients (70.0 ± 7.5 years; 6 males and 4 females) and 8 diabetic (DM) patients (65.0 ± 7.4 years; 4 males, 4 females) were dialyzed with CL-PS. After that, we treated all of the patients with CL-EE for six months. Malondialdehyde (MDA), advanced glycation end products (AGEs), and 8-hydroxydeoxyguanosine (8-OHdG) were monitored as biomarkers for oxidative stress at the start and then at one, three, and six months into treatment with CL-EE.

Results. Serum MDA, AGE, and 8-OHdG levels increased after the hemodialysis with CL-PS. The increase of the biomarkers was completely prevented by a single use of CL-EE. Long-term hemodialysis with CL-EE for six months significantly reduced the basal levels of the oxidant markers at one month for AGE and at six months for 8-OHdG in both DM and NDM patients. Serum MDA was reduced in only DM patients at three months. The improvement of the oxidative stress with CL-EE was more prominent in the DM patients.

Conclusions. Long-term treatment with CL-EE efficiently improves the oxidative stress associated with hemodialysis and potentially reduces dialysis complications due to oxidative stress.

The generation of oxygen-free radicals is a major pathogenic factor for tissue damage in many clinical conditions [1]. Levels of oxygen-free radicals are normally kept in check by an array of biochemical defense mechanisms, which include enzyme scavengers and antioxidant trappers. Oxidative stress results when the balance between oxidant production and antioxidant activity shifts in favor of the former [2, 3].

Clinical studies have confirmed that hemodialysis (HD) is associated with the development of oxidative stress and disturbance in the enzyme systems that protect against oxygen free radicals [4, 5]. The antioxidative reserve of uremic patients is significantly lower than in normal subjects [6]. Moreover, HD is associated with the activation of polymorphonuclear leukocytes, which are a major source of oxidants in vivo. When activated, these cells exhibit a burst of oxygen consumption and produce a variety of reactive substances, including superoxide radicals, hydrogen peroxide, and hypochlorous acid [7]. Accelerated oxidative stress is a pathogenic factor in some common HD-related side effects such as anemia, defective immunologic and coagulative functions, accelerated atherosclerosis and aging, β_2-microglobulin (β_2m) amyloid arthropathy, and carcinogenesis [8]. While synthetic membranes have significantly improved biocompatibility, the problem of oxygen-free radical production during HD remains largely unresolved [9], probably because of the insufficient level of biocompatibility achieved and the multiple mechanisms involved in oxygen free radical production [8].

Because vitamin E acts as a powerful hydrophilic scavenger to protect plasma lipids and cell membranes from peroxidative events [10], the use of HD filters with vitamin E-coated surface was proposed as a novel approach to reduce the accelerated generation of reactive oxygen species. Previous studies have suggested that a vitamin E-coated membrane is a highly biocompatible material, the antioxidant properties of which can exert a site-specific and timely scavenging function against oxygen-free radicals in synergy with a hypostimulatory action on the polymorphonuclear respiratory burst [11–14].

The present study was designed to elucidate the potential benefits of a new vitamin E-coated membrane on oxidative stress compared with polysulfone membrane

Key words: advanced glycation end-products, malondialdehyde, 8-hydroxydeoxyguanosine, diabetes mellutus, antioxidant.

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Table 1. Clinical features of the study groups

Group	Non-diabetic patients	Diabetic patients
N (male/female)	10 (6/4)	8 (4/4)
Age *years*	70.5 ± 7.5	65.0 ± 7.4
Dry wt *kg*	52.5 ± 5.5	48.2 ± 5.8
HbA1c %	ND	6.4 ± 0.7
Duration of dialysis *months*	58.4 ± 7.2	57.3 ± 14.1
Mean effective blood flow *mL/min*	204.0 ± 18.4	197.5 ± 12.8

Data are mean ± SD. Abbreviations are: HbA1c, hemoglobin A1c; ND, not done. All patients were treated with standard bicarbonate HD at a flow rate of 500 mL/min from a central supply system. They were dialyzed three times weekly for 4 hours and stabilized in a condition of good clinical metabolic equilibrium. Dialyzers were not reused.

by measuring the levels of malondialdehyde (MDA), advanced glycation end products (AGE), and 8-hydroxydeoxyguanosine (8-OHdG) as indicators. We also elucidated the long-term effects of this new generation of biomaterials on the correction of oxidative damage in HD. Our results confirmed that oxidant stress could be prevented, or at least reduced, by using a vitamin E-coated membrane.

METHODS

Patients

Ten nondiabetic (NDM) patients, aged from 59 to 77 years (70.0 ± 7.5 years; mean ± SD, 6 males and 4 females), and 8 diabetic (DM) patients, aged from 52 to 72 years (65.0 ± 7.4 years; 4 males and 4 females) undergoing maintenance HD for more than three years were enrolled in this study (Table 1). Patients with any of the following conditions were excluded from the study: smokers, if they were taking antioxidants, had acute or chronic infections, evidence of malignancy, or those with considerable iron overload or rheumatological disorders. The duration of dialysis was from 50 to 67 months (58.4 ± 7.2 months) in NDM patients and from 37 to 78 months (57.3 ± 14.1 months) in DM patients. Blood glucose in all DM patients was controlled by a subcutaneous insulin injection. Dialyzers were not reused. We used bicarbonate dialysate at a flow of 500 mL/min from a central supply system. Endotoxin levels in the dialysate had been below the detection limit (<1.0 pg/mL) during the observation period. All patients were dialyzed three times weekly for four hours and stabilized in a condition of good clinical metabolic equilibrium. Informed consent was obtained from all patients. At the time of sample collection, no patients had any complications. For at least three months before beginning the protocol, all patients were dialyzed with a polysulfone 1.2 to 1.5 m^2 hollow fiber dialyzer, Clirans® PS (CL-PS; Terumo Corp., Tokyo, Japan). HD, using a vitamin E-coated regenerated cellulose 1.2 to 1.5 m^2 hollow fiber dialyzer, Clirans® EE-N (CL-EE; Terumo Corp.) was then commenced. After six months of using the CL-EE filter, the dialyzer was exchanged for CL-PS again.

Blood sampling

Blood was withdrawn at the time of routine laboratory investigations on the first treatment day of the first week of every month. Blood was collected from the arterial line just before HD treatment on four occasions per patient: before (0 month) and after one, three, or six months of using CL-EE membrane. Furthermore, blood was taken at three months after changing the dialyzer back to the CL-PS membrane (9 months). Before using the CL-EE membrane (0 month) and at the first HD with the CL-EE membrane, a blood sample was withdrawn from the arterial line before and after HD treatment for evaluation of the elimination rate. Blood samples were immediately mixed with butylated hydroxytoluene to prevent further oxidation and with potassium-ethylenediaminetetraacetic acid (K-EDTA). Blood samples were centrifuged, separated into cryotubes, and stored frozen at −80°C within 60 minutes of collection until assay. Each assay was carried out on duplicate samples.

Assays

Serum AGE concentrations were measured by a newly developed enzyme-linked immunosorbent assay (ELISA) method using anti-AGE keyhole limpet hemocyanin, which mainly recognizes carboxymethyllysine (CML). The concentrations of MDA were evaluated from the reaction resulting in the formation of thiobarbituric acid reactive substances according to method of Yagi [15]. Briefly, lipids and proteins were precipitated using 10% phosphotungistic acid and N/12 sulfuric acid. The sediment was resuspended in distilled water followed by the addition of thiobarbituric acid. The reaction mixture was heated at 95°C for 60 minutes; thiobarbituric acid (TBA)-reacting substances were extracted with butanol. After centrifugation, the butanol layer was taken for fluorometric measurement at 515 nm excitation and 550 nm emission. The concentration of 8-OHdG was determined using commercially available competitive ELISA kits (8-OHdG Check; Japan Institute for Control of Aging, Fukuroi, Shizuoka, Japan). The kit can measure 8-OHdG values ranging from 0.64 to 2000 ng/mL. The specificity of the monoclonal antibody N45.1 used in the competitive ELISA kit has previously been established [16]. Measurement of serum β_2m was carried out by radioimmunoassay, an enzymatic method. Routine clinical parameters (total protein, albumin, total cholesterol, hemoglobin, and hemoglobin A1c) were determined by automatic analyzer at the clinical laboratory, Hiroshima Prefectual Welfare Federation of Agricultural Cooperative, Fuchu General Hospital. Dialysis dose was calculated as double-pool Kt/V according to Daugirdas and Ing [17]. The percentage of reduction in the serum concentration of

Table 2. Routine clinical parameters measured during the study

Filter type	0 month CL-PS	1 month	3 months	6 months	9 months CL-PS
		CL-EE			
Total protein *g/dL*					
NDM	6.54 ± 0.21	6.58 ± 0.20	6.66 ± 0.29	6.62 ± 0.20	6.58 ± 0.34
DM	6.38 ± 0.26	6.33 ± 0.55	6.43 ± 0.42	6.44 ± 0.56	6.38 ± 0.19
Albumin *g/dL*					
NDM	3.90 ± 0.28	3.86 ± 0.22	3.92 ± 0.18	3.91 ± 0.29	3.84 ± 0.30
DM	3.75 ± 0.05	3.69 ± 0.19	3.80 ± 0.13	3.83 ± 0.21[a]	3.68 ± 0.25
Total cholesterol *mg/dL*					
NDM	149 ± 36	156 ± 35	168 ± 30[a]	164 ± 46[a]	155 ± 40
DM	157 ± 29	162 ± 25	157 ± 35	172 ± 38[a]	156 ± 22
Hemoglobin *g/dL*					
NDM	10.4 ± 0.9	10.4 ± 0.6	10.4 ± 0.3	10.2 ± 0.4	10.2 ± 0.8
DM	10.4 ± 1.1	10.0 ± 0.8	10.0 ± 0.9	10.0 ± 0.6	10.2 ± 1.4
EPO *U/kg/week*					
NDM	75.9 ± 43.3	76.0 ± 43.3	70.1 ± 43.1	71.4 ± 31.3	84.2 ± 13.8
DM	104.4 ± 61.3	94.8 ± 75.3	93.6 ± 70.9	111.7 ± 66.3	103.5 ± 77.1
Kt/V					
NDM	1.30 ± 0.11	1.35 ± 0.14	1.32 ± 0.15	1.33 ± 0.16	1.33 ± 0.16
DM	1.35 ± 0.15	1.33 ± 0.17	1.41 ± 0.24	1.31 ± 0.19	1.32 ± 0.18

Data are mean ± SD. Abbreviations are: CL-PS, polysulfone dialyzer; CL-EE, vitamin E-coated regenerated cellulose dialyzer; EPO, erythropoietin; NDM, non-diabetic patient group; DM, diabetic patient group.

[a] $P < 0.05$ vs. 0 month

each parameter was calculated as follows: [(serum concentration predialysis − serum concentration postdialysis)/predialysis] × 100 (%).

Statistical analysis

Data are expressed as mean ± SD. Differences between groups were examined for statistical significance using the Mann–Whitney Rank Sum test. A P value of less than 0.05 was denoted the presence of a statistically significant difference.

RESULTS

Table 2 shows various clinical parameters of patients participating in the study. There was no change in serum total protein level between before (0 month) and after use of the CL-EE membrane for six months in either group. Serum albumin was significantly elevated in the DM group after using the CL-EE membrane for six months ($P < 0.05$). In the NDM group, the total cholesterol level was significantly higher after three months of treatment with the CL-EE membrane than the baseline level ($P < 0.05$). Similarly, in the DM group, total cholesterol level was significantly higher after six months of treatment with the CL-EE membrane than the baseline level ($P < 0.05$). After changing the membrane back to CL-PS, albumin levels in the DM group and total cholesterol levels in both groups returned to the baseline levels. During six months of treatment with the CL-EE membrane, hemoglobin levels and doses of erythropoietin required to maintain the hemoglobin level remained stable. Kt/V, a parameter reflecting the effect of HD, did not change during the observation periods.

Predialysis and postdialysis serum concentrations of MDA using CL-PS or CL-EE membranes are shown in Table 3. Before HD, serum MDA concentrations were lower in the NDM group compared with the DM group ($P < 0.01$) with both CL-PS and CL-EE membranes. HD with CL-PS membranes were associated with a significant rise in serum MDA levels in the DM group ($P < 0.05$ vs. predialysis). In the NDM group, serum MDA levels did not change during a single HD with CL-PS. In contrast, after a single HD session with CL-EE, serum MDA levels significantly decreased compared with predialysis in both the NDM ($P < 0.01$) and DM groups ($P < 0.05$). The reduction rates were significantly higher during HD with CL-EE membranes compared with HD with CL-PS membranes in both groups ($P < 0.05$). Figure 1 shows changes in serum MDA levels during the course of dialysis treatment with CL-EE membranes. Before using CL-EE membranes, serum MDA levels were higher in the DM group than in the NDM group ($P < 0.01$). One month of treatment with the CL-EE membrane did not change MDA levels in both groups. After three months of treatment with CL-EE, the MDA concentration decreased significantly in patients of the DM group ($P < 0.01$ vs. 0 month); however, NDM patients did not show any significant reduction even after six months. Serum MDA concentrations tended to increase marginally in the DM group after changing back to the CL-PS membrane.

Advanced glycation end product concentrations, both predialysis and postdialysis, were significantly lower in the NDM group compared with the DM group (Table 3). Serum AGE levels were significantly higher after

Table 3. Reduction rates of various parameters during a single hemodialysis session

	CL-PS			CL-EE		
	Predialysis	Postdialysis	RR %	Predialysis	Postdialysis	RR %
MDA *nmol/mL*						
NDM	3.00 ± 0.31^{b}	3.02 ± 0.28^{b}	-1.1 ± 8.4	2.96 ± 0.60^{b}	2.83 ± 0.59^{d}	9.8 ± 4.3^{e}
DM	3.62 ± 0.27	4.10 ± 0.37^{c}	-12.9 ± 13.9	3.58 ± 0.24	3.29 ± 0.19^{c}	5.0 ± 7.4^{e}
AGE *IU/mL*						
NDM	24.8 ± 4.6^{b}	$30.5 \pm 5.2^{b,d}$	-24.1 ± 12.6	25.8 ± 2.2^{b}	25.7 ± 5.0^{a}	2.3 ± 16.0^{f}
DM	38.0 ± 8.9	45.9 ± 12.4^{d}	-20.0 ± 10.5	39.2 ± 9.3	39.1 ± 12.4	0.6 ± 31.8^{e}
8-OHdG *ng/mL*						
NDM	102.8 ± 78.9	135.8 ± 99.5^{d}	-32.6 ± 29.5	116.7 ± 94.3	122.9 ± 94.4	-5.3 ± 16.2^{e}
DM	230.4 ± 193.3	264.1 ± 253.1	-15.1 ± 27.9	226.4 ± 143.8	215.9 ± 224.5	4.6 ± 12.7^{f}
β_2m *mg/mL*						
DM	26.8 ± 7.1	11.0 ± 3.4^{d}	59.7 ± 3.3^{a}	27.0 ± 7.2	25.6 ± 7.04	5.4 ± 6.6^{f}
DM	28.3 ± 3.9	12.6 ± 2.2^{d}	55.4 ± 3.1	28.2 ± 6.4	26.7 ± 6.9^{e}	5.6 ± 5.7^{f}

Data are mean ± SD. Abbreviations are: reduction rate (RR) was calculated as described in the **Methods** section. CL-PS, polysulfone dialyzer; CL-EE, vitamin E-coated regenerated cellulose dialyzer; MDA, malondialdehyde; AGE, advanced glycation end products; 8-OHdG, 8-hydroxy-deoxyguanosine; β_2m, β2-microglobulin; NDM, non-diabetic patient group; DM, diabetic patient group.

[a] $P < 0.05$, [b] $P < 0.01$ vs. DM

[c] $P < 0.05$, [d] $P < 0.01$ vs. predialysis

[e] $P < 0.05$, [f] $P < 0.01$ vs. CL-PS

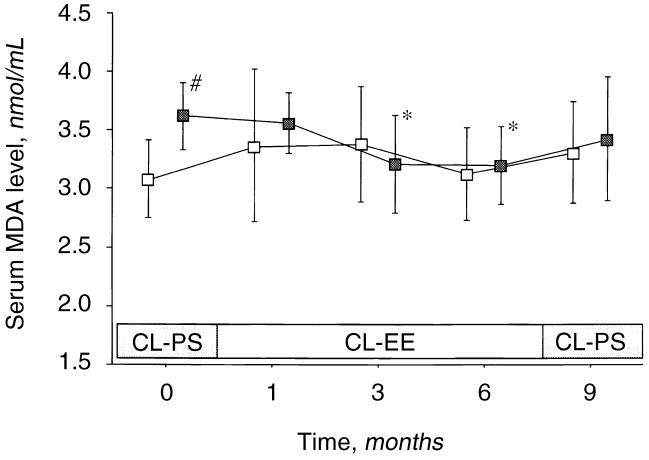

Fig. 1. Serum levels of malondialdehyde (MDA). Changes in serum MDA levels during the course of dialysis treatment with a vitamin E-coated regenerated cellulose hollow fiber dialyzer (CL-EE) membrane. At baseline, serum MDA levels were higher in the diabetic (DM; ■) group than in the non-diabetic (NDM; □) group. After three months of treatment with CL-EE, the MDA concentration significantly decreased in patients of the DM group. However, the NDM patients did not show any significant reduction even after six months. After reverting to CL-PS, serum MDA increased to original levels in the DM group. Data are mean ± SD. #$P < 0.01$ vs. NDM group; *$P < 0.01$ vs. baseline level.

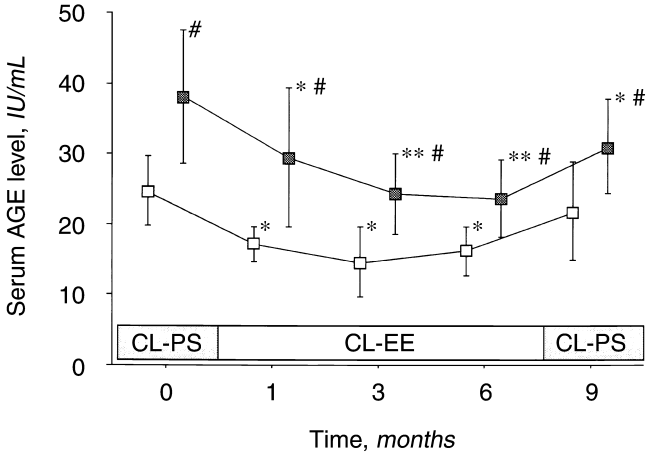

Fig. 2. Serum levels of advanced glycation end products (AGEs). Changes in serum AGE levels during the course of dialysis treatment with CL-EE membrane are shown. Serum AGE levels were significantly higher in the DM group (■) than in the NDM group (□) at all observation points. After one month of treatment with CL-EE, a significant reduction in the AGE concentration was noted in both groups. After three months of CL-EE use, there was a greater decrease in AGE in the DM group than the NDM group. After reverting to CL-PS, serum AGEs in the NDM group returned to baseline levels. Data are mean ± SD. #$P < 0.01$ vs. NDM group; *$P < 0.05$ and **$P < 0.01$, respectively, vs. baseline levels.

dialysis with the CL-PS membrane in both groups ($P <$ 0.01 vs. predialysis), whereas no difference in these levels was observed using the CL-EE membrane in either group. The percentage of reduction in AGE concentration with CL-EE membrane was higher compared with CL-PS membrane ($P < 0.01$, NDM group; $P < 0.05$, DM group). Serum AGE levels were significantly higher in the DM group than in the NDM group at all of the time intervals ($P < 0.01$; Fig. 2). A significant progressive decrease in AGE concentrations was observed in both the NDM and DM groups ($P < 0.05$ vs. 0 month, $P <$ 0.01 vs. 0 month). After reverting to the CL-PS membrane, serum AGE concentrations in the NDM group returned to original levels, whereas serum AGE concentrations in the DM group tended to increase but not to the original pre-CL-EE membrane dialysis level.

Reduction rates in 8-OHdG concentrations during a single HD session are shown in Table 3. During CL-PS membrane use, 8-OHdG increased during a single HD session. In contrast, no significant increase of 8-OHdG

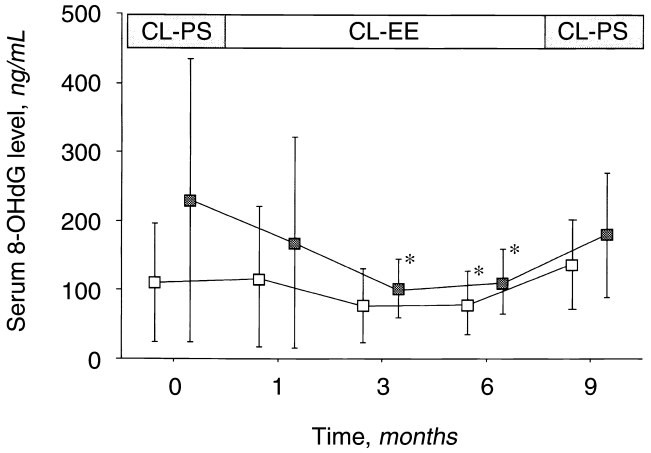

Fig. 3. Serum levels of 8-hydroxy-deoxyguanosine (8-OHdG). Changes in serum 8-OHdG levels during the course of dialysis treatment with CL-EE membrane are shown. A significant reduction was noted in the DM group (■) by three months of treatment with CL-EE. Serum 8-OHdG levels also significantly decreased in the NDM group (□) after six months of treatment. After reverting to CL-PS membrane, serum 8-OHdG tended to increase in both groups. Data are mean ± SD. *$P <$ 0.05 vs. baseline levels.

was observed during CL-EE membrane use. The percentage of reduction in 8-OHdG concentration with CL-EE membrane was significantly higher than with CL-PS membrane ($P < 0.05$, NDM group; $P < 0.01$, DM group). Figure 3 shows changes in serum 8-OHdG levels during the course of dialysis with CL-EE membranes. In the DM group, serum 8-OHdG levels were significantly reduced by three months of treatment with CL-EE ($P <$ 0.05 vs. 0 month). After 6 months of treatment, serum 8-OHdG levels in the NDM group were also significantly decreased ($P < 0.05$ vs. 0 month). Serum 8-OHdG levels tended to increase in both groups after reverting to CL-PS membrane.

The percentage of reduction in β_2m concentrations during a single HD session is shown in Table 3. The reduction rate in NDM patients using the CL-PS membrane was higher than in DM patients ($P < 0.05$). Reduction rates were significantly lower in patients using the CL-EE membrane than the CL-PS membrane in both groups ($P < 0.01$). After several months of CL-EE membrane use, serum β_2m significantly and progressively increased, compared with the values measured before CL-EE membrane use in both the NDM and DM groups (data not shown). After changing back to the CL-PS membrane, serum β_2m concentrations returned to the levels noted prior to CL-EE membrane use.

DISCUSSION

Oxygen-free radicals and other reactive molecules can affect the integrity of virtually all biomolecules, including lipids, proteins, and nucleic acids, ultimately leading to cell damage. Consistent observations have provided evidence for the presence of oxidative stress in chronic renal failure patients, particularly in HD. HD is accompanied by activation of polymorphonuclear leukocytes, which are the major source of oxidants in vivo. When activated, these cells exhibit a burst of oxygen consumption and produce a variety of reactive substances, including superoxide radicals, hydrogen peroxide, and hypochlorous acid [7]. Use of a bioincompatible dialysis system results in a dramatic increase in the production of reactive oxygen species, thus reducing antioxidant defense mechanisms. In recent years, the biocompatibility of synthetic membranes has been markedly improved. However, the problem of an oxidative stress resulting from HD remains largely unresolved [9]. Previous studies suggested that vitamin E-modified filters could be effective in protecting HD patients against damage caused by oxygen-free radicals by decreasing the activation of polymorphonuclear leukocytes and providing a better control of blood lipoperoxidation and antioxidant status [11]. In this study, we evaluated the long-term effects of a new generation of biomaterials on the correction of oxidative damage to lipids, proteins, and nucleic acids during HD.

Endotoxin in dialysate is one possible source of reactive oxygen species in HD patients. Cytokine release by neutrophils is enhanced by endotoxin [18], which produces a variety of reactive substances, including superoxide radicals, hydrogen peroxide, and hypochlorous acid. We have used endotoxin cut filters to provide clean dialysate, such that endotoxin levels in dialysate were controlled to maximum detection sensitivity. Endotoxin was considered to have no effect on HD patients by dialysate back filtration.

Serum albumin was significantly elevated in the DM group after using the CL-EE membrane at six months. After changing the membrane back to CL-PS, albumin levels in the DM group returned to the baseline levels (see Table 2). In general, there is much more amount of albumin leak in polysulfone membrane compared with cellulose membrane. We considered that the albumin leak was improved yet a little by changed to the CL-EE membrane that is modified from cellulose based low-flux membrane. The total cholesterol level in the both groups significantly increased after treatment with the CL-EE membrane than the baseline level (see Table 2). A previous study has reported that there was no difference between high- and low-flux dialysis for the total cholesterol [19]. It is not unclear why total cholesterol levels increase, but it might be related to the decline of the albumin level.

During the observation periods using CL-EE membranes, hemoglobin levels and doses of erythropoietin required to maintain the hemoglobin level remained stable (see Table 2). It has been suggested that oxidative stress could be one resistance factor to erythropoietin

response during HD and that vitamin E supplementation could have a sparing effect on erythropoietin dose requirements [20]. The use of vitamin E-modified dialysis filters demonstrated that such filters could improve erythrocyte stability and therefore HD-related anemia [21]. The effects of CL-EE on chronic hemolysis in HD patients could not be confirmed in our study as we used CL-PS, a polysulfone membrane with a fine biocompatibility [22], as a control. Polysulfone membranes are known to be good biocompatible dialyzer membranes. Previous studies reported that the polysulfone dialyzer increased the albumin level and decreased the activation of leukocytes, platelets, and monocytes [23–25]. Furthermore, HD using a high-flux polysulfone dialyzer was associated with a low pentosidine plasma level, a surrogate marker for carbonyl precursors [26]. However, it has been reported that in vitro, vitamin E-modified membranes induce less oxidative stress in polymorphonuclear cells compared with polysulfone [27]. Even though a crossover method was applied, time or other effects could not be completely ruled out in our study. Nevertheless, our results also suggest that in vivo, CL-EE, vitamin E-coated membranes have better biocompatibility with regard to antioxidative effects than the CL-PS polysulfone membranes.

Lipid peroxidation products such as MDA have been identified in atherosclerotic lesions [28, 29]. Oxidatively modified lipoprotein is recognized by scavenger receptors and taken up by macrophages, inducing the development of foam cells in vascular atheroma [30]. Several groups have described the presence of elevated levels of lipid peroxidation products in HD patients [31–34]; this is consistent with the finding that patients on long-term dialysis develop atherosclerosis sooner than age-matched controls [35, 36]. In the present study, we have demonstrated that serum MDA levels significantly decreased after a single HD session with CL-EE compared with predialysis (see Table 3) and that patients of the DM group exhibited a significant reduction in MDA concentration during six months of observation (see Fig. 1). Because of its direct protective effect against lipid peroxidation, vitamin E appears to be an appropriate agent to reduce HD-induced oxidative stress [18]. The long-term use of CL-EE might therefore reduce the risk of dialysis-related atherosclerosis.

The concentration of AGE-modified proteins increases during the aging process, as well as in diabetes and uremia, resulting in tissue damage through alterations of their structure and function [37]. AGEs in DM patients have been reported at a higher level than in NDM patients [38]. The present study also found that HD patients with DM exhibited higher serum AGE levels than NDM patients (see Fig. 2), suggesting that DM patients receiving HD were exposed to higher oxidative stress. AGE generation in patients with uremia is stimulated by accumulation of carbonyl intermediates resulting from oxidation of glucose and ascorbic acid [39]. AGE-modified proteins have been identified in atherosclerotic lesions of the human aorta [40]. Therefore, the accumulation of AGE may be one factor that is responsible for cardiovascular complications in maintenance dialysis patients. In the present study, the use of CL-EE membranes decreased serum AGE levels in both the NDM and DM groups (see Fig. 2). Together with the fall in MDA action, CL-EE may suppress the progression of HD related atherosclerosis.

β_2-Microglobulin is a major protein constituent of the amyloid fibrosis in HD-related amyloidosis, a common complication among long-term HD patients [41]. The serum β_2m level significantly and progressively increased with the CL-EE membrane because of a lower reduction rate compared with the polysulfone membrane (see Table 3). The differences in filtration of β_2m between both membranes are that the polysulfone dialyzer is a high-flux membrane with a large pore size, while the backbone of the CL-EE dialyzer is a low-flux cellulose membrane. Therefore, the serum β_2m level became increased by the use of the CL-EE membrane. However, it is well known that there is no statistical correlation between its serum concentration and the occurrence of the HD-related amyloidosis [42], and the accumulation of AGE in amyloid tissue is thought to be the cause of HD-related amyloidosis [43]. The AGE-modified proteins have been shown to stimulate monocyte/macrophage to secrete bone-resorbing cytokines so that the modification of bone matrices with AGE might play a pathophysiological role in HD-related amyloidosis [44]. The use of CL-EE membranes significantly decreased serum AGE levels so that there is some possibility of reducing the AGE-β_2m level. Further long-term studies are needed to clarify this issue.

It has been suggested that aggregation of DNA damage is relevant to the aging process and the incidence of cancer [45]. Oxidative stress is known to increase the conversion of deoxyguanosine to 8-OHdG in DNA, and therefore, 8-OHdG levels are thought to be a marker of oxidative DNA damage [46]. Increased excretion of urinary 8-OHdG is observed in patients with non–insulin-dependent diabetes mellitus [47], atopic dermatitis [48], and lung cancer [49]. Previous studies have demonstrated that 8-OHdG decreased in a time-dependent manner after supplementation with vitamin E [50]. Our study demonstrates, to our knowledge for the first time, that the use of CL-EE prevented the production of 8-OHdG during a single HD (see Table 3) and that the level of 8-OHdG was gradually decreased by the long-term use of CL-EE, particularly in DM patients (see Fig. 3). We infer from these results that oxidative damage to DNA was diminished.

The CL-EE membrane can specifically interact with

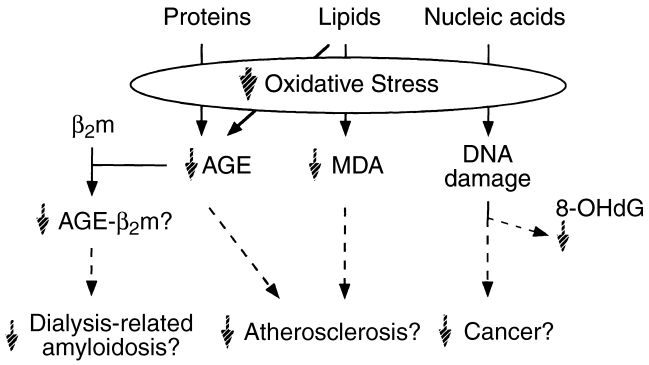

Fig. 4. Effects of CL-EE on HD-related complications. Schematic diagram of the potential beneficial effects of CL-EE on HD-related complications.

the dynamic concentration and distribution equilibrium of endogenous antioxidants in the blood, and since vitamin E is a normal constituent of cell membranes and plasma, it can supplement these defenses and protect against lipoperoxidation [11]. The CL-EE membrane can exert a site-specific and timely scavenging function against oxidative-free radicals, in synergy with its effects of decreased leukocyte activation and oxidative-free radical production. Other clinically relevant effects of this vitamin E-modified membrane include decreased platelet activation, together with the anti-apoptogenic effect on mononuclear leukocytes, restoration of phagocyte function [11], and inhibitory function of acute cytokine production during dialysis treatment [12].

In conclusion, the results of the present study indicate that CL-EE is more effective than CL-PS in diminishing oxidative stress to all biomolecules, including lipids, proteins, and nucleic acids, during HD, especially in DM patients. HD using a CL-EE membrane may therefore protect HD patients against oxidative damage and could reduce dialysis-related amyloidosis, atherosclerosis, cancer risk, and other diseases caused by free radicals associated with HD (Fig. 4). Further longitudinal studies in patients on long-term dialysis should be undertaken to clarify the potential benefits of antioxidant therapy in end-stage renal failure patients.

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